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Case reports

Neurovisceral ceroid-lipofuscinosis in blind Devon cattle*

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Summary. Ten Devon cattle from a single property were affected with blindness from 14 months of age. Severe retinal degeneration progressing to atrophy was associated with widespread intracellular accumulation of pigment in the retinal ganglion cells, central nervous system and major organs. The pigment was consistent with ceroid-lipofuscin granules on histological, histochemical and ultrastructural examination. Although a familial relationship existed between affected individuals, a pattern of inheritance could not be established by examination of available breeding records. The disease is compared to similar disorders reported in man and other species.

Key words: Devon cattle – Blindness – Retinal atrophy – Ceroid-lipofuscinosis – Batten disease

Lipofuscin granules are derived from lysosomes and other intracellular organelles, and are composed of an autofluorescent pigment and an associated lipid component that present as lipopigment complexes under electron microscopy [3]. Neurones of the central nervous system accumulate lipofuscin throughout life, however in certain disorders known as the ceroid-lipofuscinoses, there is excessive intracellular accumulation of fluorescent granules of lipofuscin or its variant ceroid, resulting in loss of functional neurocytoplasm [19]. These diseases have been described in man (Batten disease) [20], South Hampshire sheep [21], Siamese cats [10] and many breeds of dogs, including English setters [18], Chihuahuas [24], Cocker spaniels [11, 22], Dachshunds [5, 27], Salukis [1], Dalmations [9], a Blue Heeler [4], a cross-bred terrier [14], and in Border Collies [26].

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Blindness is an important feature of the disease in man, sheep [2] and some breeds of dogs. This paper records the occurrence of blindness in Devon cattle due to retinal atrophy associated with widespread and severe neuronal and some visceral accumulation of ceroid-lipofuscin.

Clinical findings

Seven Devon cows and three steers were detected with blindness over a 10-year period on a property in the southern tablelands region of New South Wales, Australia.

Clinical signs were observed from 14 months of age. Affected animals collided with obstacles, and tended to walk or trot in a circle when disturbed, frequently with a mild head tilt. They maintained good condition except in periods of shortage of pasture feed, when they became weak and demonstrated a paretic gait. Most eventually died from misadventure within 2 years of the onset of blindness. Indirect opthalmoscopic examination of two cows demonstrated increasingly severe retinal degeneration with age, with decreased reflectivity of the tapetum, optic disk pallor and depigmentation of the non-tapetum.

Gross findings

Five affected cows and one steer were examined in detail. The animals were slaughtered, a necropsy performed, and specimens from many organs including the brain, spinal cord and optic nerves were prepared routinely for microscopic and ultrastructural examination. At post-mortem, the brain of one cow appeared slightly smaller than normal. A yellow-brown discolouration of the cerebrocortical grey matter was noted in this cow and similar discolouration was present to a lesser extent in the white matter of the brain, the renal pelvis and the serosa of the upper small intestine.

Histological findings

On microscopic examination, the eyes of all the affected animals exhibited severe retinal atrophy characterised by complete loss of the layer of rods and cones, and loss of the outer limiting membrane (Fig. 1). Degeneration of the outer nuclear layer, inner nuclear layer, inner plexiform layer, and nerve fiber layer was present, with some loss of the ganglion cells. Most of the

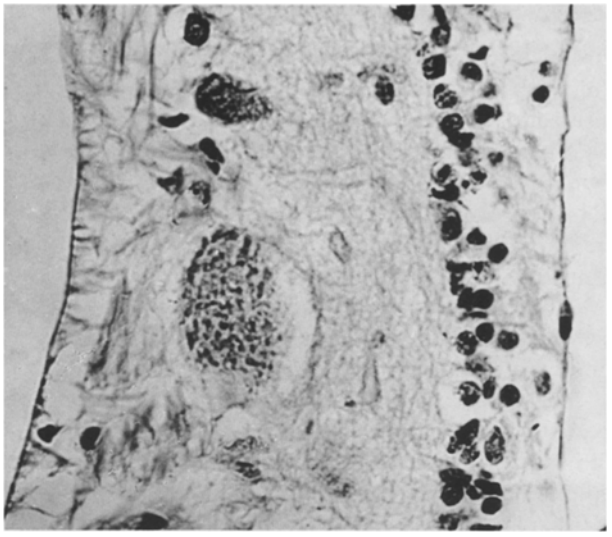

Fig. 1. Retina. Atrophy of the layer of rods and cones and accumulation of granules in the cytoplasm of a retained ganglion cell. Luxol fast blue-PAS; ×328

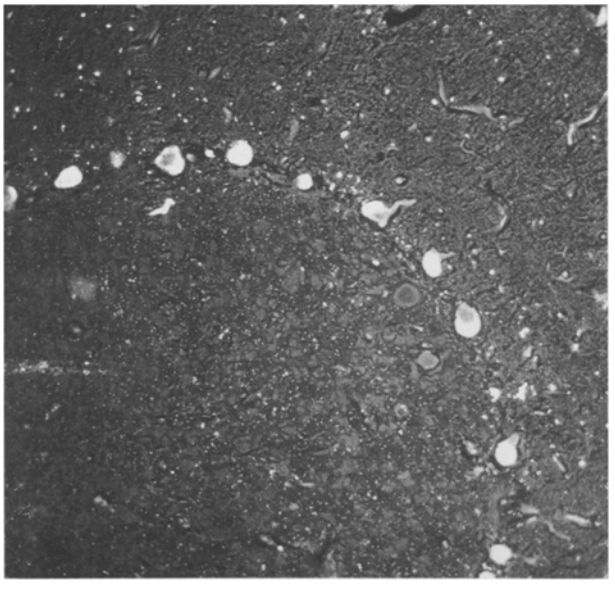

Fig. 2. Cerebellum. Fluorescent pigment in purkinje cell cytoplasm. Unstained paraffin section, ×164

retained ganglion cells were distended by prominent aggregates of granular cytoplasmic inclusions. No lesions were apparent in the optic nerves or retinal pigment epithelium.

Intracytoplasmic accumulation of granules was noted in many areas of the body, particularly in the central nervous system (CNS). The granules were irregularly shaped to spherical. Considerable variation was observed in their number between affected cells, however most aggregates contained in excess of 10 separately identifiable granules. In cells containing less storage material, the granules were mostly grouped together at the periphery of the cytoplasm. However in cells with abundant granules, the nucleus was peripherally located and the cytoplasm contained an almost confluent perinuclear halo of coalescing granular material. The granules were not observed to extend beyond the axon hillock of CNS neurones. The granules stained light brown to red with H&E, magenta with PAS, bright blue with luxol fast blue (LFB) and were acid-fast. A brilliant yellow-green autofluorescence was observed in the cytoplasm of cells containing granules when compared to control tissues (Fig. 2). In toluidine blue sections, many granules appeared to be hollow spheres.

Within the CNS, there was mild loss of laminar neurones in some regions of the cerebral cortex. Intracytoplasmic granule accumulation was evident in many of the retained neurones and occasional glial cells. In the cerebellum, the purkinje cells and some cells of the molecular layer were distended by granular aggregates, and in some foliae there was degeneration and loss of purkinje cells and mild depletion of cells from the granular cell layer. Occasional eosinophilic axonal swellings (spheroids) were present in the cerebellar white matter tracts with more numerous axonal swellings present adjacent to large granule-affected neurones of the cerebellar roof nuclei, mid-brain and brain stem nuclei. Areas of more severe axonal degeneration were frequently accompanied by mild gliosis. In the spinal cord, prominent intracytoplasmic granule accumulation was present in neurones, particularly of the ventral horn grey matter. Few axonal swellings were noted in the spinal cord, however occasional axons appeared to be undergoing Wallerian-type degeneration in the lateral and ventral white matter funiculi. Mild to moderate granule accumulation was present in neurones of the dorsal root and gasserian ganglia, however no lesions were observed in the axons of the peripheral nerves or in the skeletal muscle.

In all affected animals there was mild to moderate accumulation of brownish intracytoplasmic granules, similar to those in neurones in the renal cortical tubular epithelium. In the liver, granule accumulation occurred in some hepatocytes, which appeared swollen and of increased eosinophilia, particularly surrounding central veins. In addition there was prominent accumulation of granules in histiocytic cells of the lymph nodes, involving the medullary sinuses and lymphoid follicles. Several lymphoid follicles contained necrotic cells forming central areas of mineralisation in one animal. Histiocytic cells containing granules were also present in the spleen and were readily distinguished from those distended with haemosiderin.

Ultrastructural findings

Ultrastructural examination revealed many membrane-bound cytosomes present in neurones and in retinal ganglion cells. The cytosomes consisted of pockets of curvilinear bodies (Fig. 3), or multimembranous structures arranged in irregular whorling configurations or as laminated stacks of membranes (Fig. 4). Frequent zebra pattern and occasional fingerprint-type cytosomes were observed (Fig. 5) and many were vacuolated. The surrounding cytoplasmic organelles were vesicular, and many mitochondria appeared enlarged, containing disoriented cristae.

Analysis of the available breeding records determined that all ten affected animals were the progeny of three related bulls. Two of the bulls were the grandson (H) and great-grandson (B) of a bull originally imported from the UK, and the third bull (P) was a grandson of bull B. However, despite the familial occurrence, it was not possible to define the mode of inheritance of the disease from the available records.

Using methods described [13], the activities of the following 10 lysosomal enzymes in granulocytes and lymphocytes from two affected animals were determined and found to be within

the range for normal cows: alpha mannosidase, hexosaminidase, arylsulfatase, alpha fucosidase, acid phosphatase, galactosaminidase, beta glucuronidase, alpha glucosidase, alpha galactosidase and beta galactosidase.

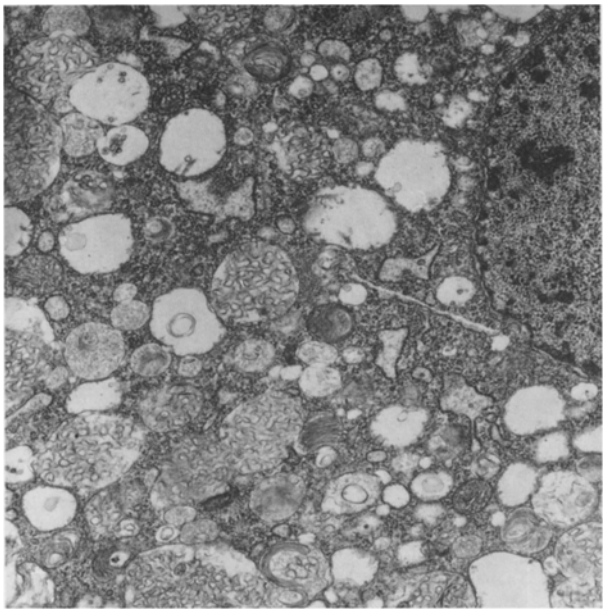

Fig. 3. Retina. Retained ganglion containing pleiomorphic membrane bound cytosomes, including curvilinear bodies, laminated membranous profiles and swollen mitochondria; × 13,432

Discussion

The histological and ultrastructural findings in this study are consistent with the animals being affected with neurovisceral ceroid-lipofuscinosis. Affected animals presented with overt blindness associated with retinal atrophy and cerebral pathology. The severe CNS neuronal accumulation of the granules and the presence of similar storage product in splenic and lymphoid histiocytes, renal tubular epithelium and hepatocytes, indicates the lesion is probably a generalised enzymic failure of metabolism resulting in systemic accumulation of membranous lipopigment.

The retinal atrophy in the Devon cattle described is associated with the accumulation of material resembling ceroid-lipofuscin granules in the preserved ganglion cells. The relationship between the accumulating material and the severe retinal degeneration is unclear. The relative retention of the ganglion cells may explain the apparently normal appearance of the optic nerves. In addition it is not known whether the onset of clinical blindness was due to the retinopathy, or to the atrophy of the optic cortex, or to concurrent lesions at both sides. In sheep it has been suggested that the central optic lesions precede the retinal changes [21].

A number of lysosomal enzyme deficiencies have been recorded in cattle, including alpha-mannosidosis

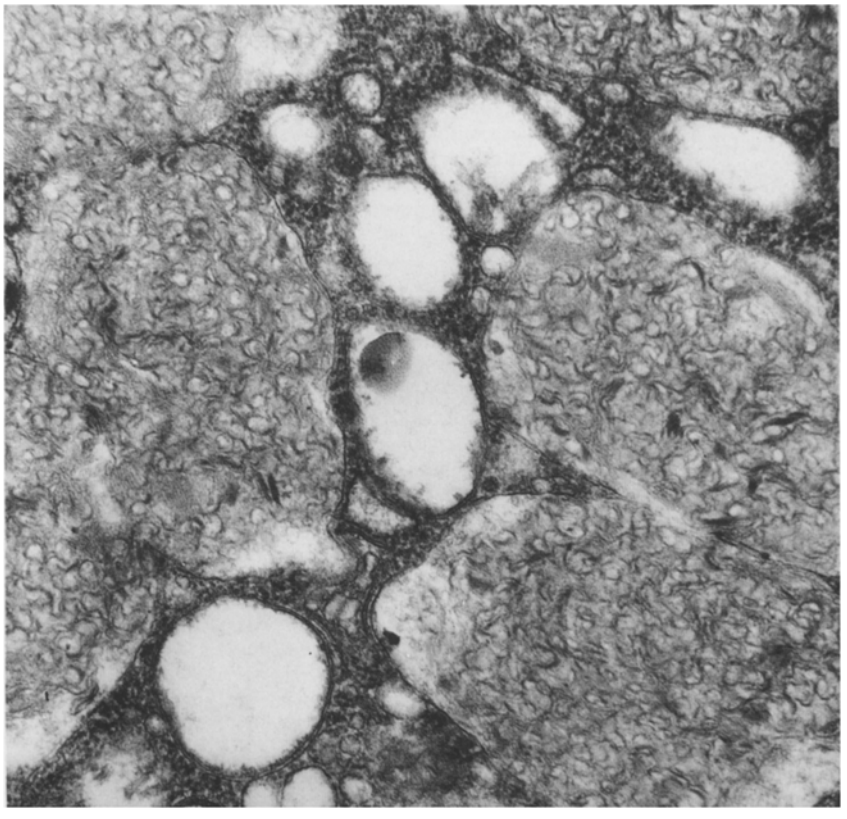

Fig. 4. Retina. Membrane-bound cytosomes containing tightly packed curvilinear bodies; × 29,200

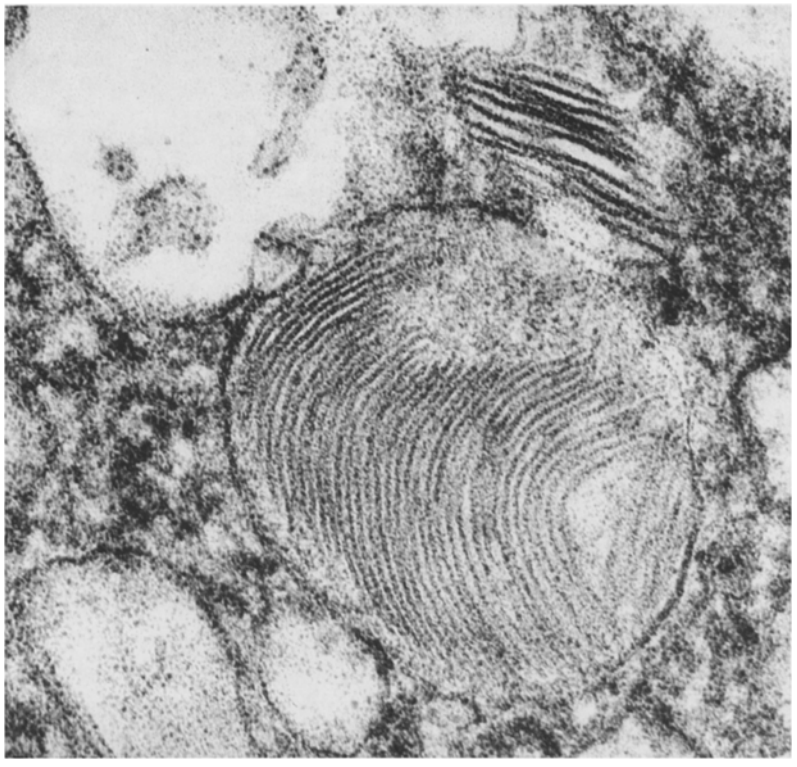

Fig. 5. Retina. Membrane-bound cytosome consisting of laminated stacks of membranes in a fingerprint pattern; ×92,000

in Angus and Murray Greys [16], generalised type II of glycogenosis in Shorthorn and Brahmans [8, 23], and GMI gangliosidosis in Friesian cattle [6]. In addition a disease described as neuronal lipodystrophy has been recorded in an inbred beefmaster bull [25] and an unidentified storage disease has been recorded in Hereford cattle [12]. These diseases are mostly identified clinically by progressive neuromuscular weakness and failure to thrive, and are readily distinguishable pathologically, despite their common morphological lesion of intracytoplasmic accumulation of storage material in the CNS or muscle. Ceroid-lipofuscinosis is Devons most closely resembles the disease recorded in the beefmaster bull [25], which was characterised by clinical blindness, and microscopic accumulation of lipid storage material that could have been ceroid-lipofuscin pigment.

Insufficient breeding information was available to draw conclusions regarding a mode of inheritance for this disorder in Devons. However, the apparent confinement of the disease to a single stud, and to progeny of three related sires used over many years would support the hypothesis that the disease is inherited. The ceroid-lipofuscinoses of man and sheep are inherited in an autosomal recessive manner [17]. The possibility of an undisclosed environmental toxic factor producing a failure of a specific lysosomal enzyme function, as recorded with interference in alpha-mannosidase activity in *Swainsona* species [7], was considered unlikely. Grazing of the plant *Trachyandra divaricata* (branched onion weed) has been associated with induced neuronal lipofuscinosis in sheep and horses in Western Australia [15], however this plant was not observed on the affected property.

The molecular pathogenesis of ceroid-lipofuscinosis is unknown. It has been determined that the fluorescent material in the form of curvilinear bodies in late infantile Batten's disease is a retinoyl complex, possibly derived from vitamin A [28]. A mitochondrial enzyme defect resulting in mitochondrial degeneration, with the mitochondrial membranes taken up by the lysosomes to form the lipofuscin granules has been suggested [2]. More recently it has been suggested that ceroid-lipofuscin complexes represent specific precursors rather than products of non-specific peroxidation mechanisms, and are derived from lysosomal and other intracellular organelles due to specific thiol endoprotease defects involved in normal organellar membrane recycling and exocytosis [29].

Until the biochemical pathogenetic mechanisms leading to the accumulation of the lipopigment storage material are resolved, the now numerous animal models of the ceroid-lipofuscinoses are likely to make valuable comparative contributions to the understanding of this group of disorders. Blindness in Devon cattle with retinal atrophy would appear to be a further interesting animal model of the neurovisceral ceroid-lipofuscinoses.

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